



Functional crosstalk between dendritic cells and Foxp3⁺ regulatory T cells in the maintenance of immune tolerance

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Peripheral immune tolerance requires a controlled balance between the maintenance of self-tolerance and the capacity to engage protective immune responses against pathogens. Dendritic cells (DCs) serve as sentinels of the immune system by sensing environmental and inflammatory signals, and play an essential role in the maintenance of immune tolerance. To achieve this, DC play a key role in dictating the outcome of immune responses by influencing the balance between inflammatory or Foxp3⁺ regulatory T (T_{reg}) cell responses. At the heart of this immunological balance is a finely regulated DC and T_{reg} cell crosstalk whereby T_{reg} cells modulate DC phenotype and function, and DC drive the differentiation of Foxp3⁺ T_{reg} cells in order to control immune responses. This review will focus on recent advances, which highlight the importance of this bidirectional DC and T_{reg} cell crosstalk during the induction of tolerance and organ-specific autoimmunity. More specifically, we will discuss how T_{reg} cells modulate DC function for the suppression of inflammatory responses and how DC subsets employ diverse mechanisms to drive differentiation of T_{reg} cells. Finally, we will discuss the therapeutic potential of tolerogenic DCs for the induction of tolerance in autoimmune diseases.

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INTRODUCTION

Immune tolerance consists of two main processes, namely central and peripheral tolerance. Central tolerance takes place in the thymus where most of the self-reactive T cells are deleted at an immature stage of their development (Bluestone, 2011). Despite negative selection, self-reactive T cells can escape thymic clonal deletion, and subsequently provoke autoimmune diseases such as type 1 diabetes (T1D), multiple sclerosis (MS), and inflammatory bowel disease (IBD) unless they are controlled by one of many peripheral mechanisms (Sakaguchi et al., 1995).

REGULATORY T CELLS ARE MAJOR MEDIATORS OF PERIPHERAL SELF-TOLERANCE

In order to ensure tolerance induction of such auto-reactive T cells in the peripheral immune system, a number of mechanisms including a network of regulatory T (T_{reg}) cells exist to achieve this function. T_{reg} cells constitute 1–10% of thymic and peripheral CD4⁺ T cells in humans and mice, and arise during a thymic selection (Sakaguchi, 2000). They are characterized by the constitutive expression of the IL-2R α chain (CD25) and expression of the forkhead winged helix transcriptional regulator Foxp3 (Hori et al., 2003). The importance of Foxp3 has been demonstrated by natural mutations of the *foxp3* gene that result in a loss of T_{reg} cell function and the development of severe autoimmune diseases, including T1D, in scurfy mice and IPEX patients (Ben-nett et al., 2001; Chang et al., 2006; d'Hennezel et al., 2009). T_{reg}

cell population can be divided into the naturally occurring Foxp3 T_{reg} population (here defined as T_{reg}), generated in the thymus and anyone of many inducible T_{reg} cell populations (here defined as iT_{reg}), that are derived in the periphery from CD4⁺Foxp3[−] precursors upon activation in presence of differentiating signals like TGF- β and IL-10 (e.g. Th3, Tr1 cells) (Haribhai et al., 2011; Pot et al., 2011).

DENDRITIC CELLS DICTATE THE BALANCE BETWEEN TOLERANCE AND IMMUNITY

Numerous studies show that different cell populations of the innate immune system such as dendritic cells (DCs), macrophages, natural killer cells, and $\gamma\delta$ T cells can regulate tolerance induction (Lehuen et al., 2010). DCs represent a heterogeneous population of bone marrow-derived cells and are the most potent antigen presenting cells (APCs) (Banchereau and Steinman, 1998; Shortman and Naik, 2007). DCs are derived from multiple lineages, have a distinct stage of development, activation, and maturation state (Banchereau and Steinman, 1998; Steinman et al., 2003). DCs exist as a distinct subset and differ in their ontogeny, surface molecule expression, and biological functions (Banchereau and Steinman, 1998; Steinman and Nussenzweig, 2002). These factors seem to determine the T cells polarizing signals and type T cells responses induced by DCs namely Th1, Th2, Th17, or T_{reg} cells. Although all DCs are able to prime T cells, they differ in their *in vivo* niches, migration, function, and requirements

from the environment for their generation and activation (Shortman and Naik, 2007). DCs are divided into conventional, myeloid, or plasmacytoid DC (pDC) subsets. In mice, three main subsets of conventional CD11c^{hi} DCs have been identified in the spleen and lymph nodes, namely the CD8⁺, CD4⁺CD8⁻, and CD4⁻CD8⁻ DCs (Vremec et al., 2000). Lymph nodes contain two additional DCs subsets; skin-derived Langerhans cells and tissue interstitial DCs that arrive from the periphery through the lymphatic circulation (Vremec et al., 2000).

Dendritic cells receive the maturation signals through the pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) receptors that detect certain microbial and tissue damage signals *via* activation of nuclear factor- κ B (NF- κ B) and interferon regulatory factors (IRF) families (Maldonado and von Andrian, 2010). Upon activation, DCs up-regulate a wide variety of gene products involved in antigen presentation and co-stimulation such as MHC II, CD86, CD80, OX40-L, inducible co-stimulator (ICOS) ligand as well cytokines involved in the modulation of effector function such as IL-1 β , IL-2, IL-6, IL-8, IL-12, and IL-18 (Maldonado and von Andrian, 2010). These changes are required for DCs to initiate a three-step T cell activation process: MHC molecules displaying cognate peptide (signal 1), co-stimulatory signal expression (signal 2), and cytokine production by DCs (signal 3).

While potentially capable to initiate inflammatory responses, DCs also play an important role in modulating tolerance induction. Tolerogenic DCs are characterized by high antigen uptake and processing capabilities in order to present antigen to antigen-specific T cells, but fail to deliver proper co-stimulatory signal for effector T (T_{eff}) cells activation and proliferation (Steinman et al., 2003). This results in T cell death, T cell anergy, or induction and expansion of T_{reg} cells subsets. As such, tolerogenic DCs have been shown to suppress experimental autoimmune disease and play an important role in alloimmunity (Morelli and Thomson, 2007).

SUBSETS OF TOLEROGENIC DCs: DIVISION OF LABOR IN TOLERANCE INDUCTION

Different DCs subsets are specialized in tolerance vs. inflammatory immune response decisions. Specific markers capable of discriminating tolerogenic from inflammatory DCs are still ill-defined. However, CD8⁺ DCs expressing CD95L and DEC205 often possess tolerogenic properties (Mahnke et al., 2002; Yamazaki et al., 2008). Expression of inhibitory immunoglobulin-like transcript (ILT) receptors is also frequently observed in some subsets of tolerogenic DCs, where ILT-3 and ILT-4 mediated signals on DCs inhibits expression of co-stimulatory molecules and induce a tolerogenic state (Manicassamy and Pulendran, 2011). In humans, ILT-3 and ILT-4 expressing tolerogenic DCs can promote antigen-specific unresponsiveness in CD4⁺ T cells and induce T_{reg} cells. Recent studies have shown that Indoleamine 2,3-dioxygenase (IDO) and IL-10 can induce the expression of ILT-3 and ILT-4 on DCs and promote tolerogenic response (Manavalan et al., 2003). Activation of ILT-3 receptor on DCs leads to the recruitment of protein phosphatase SHP-1 and SHIP-1 to the immunoreceptor tyrosine-based inhibitory motif (ITIM) resulting in the inhibition of NF- κ B and p38MAPK pathways that are critical for inflammatory responses (Cella et al., 1997; Svajger et al., 2008).

While immature DCs appear to be good indicators for DC tolerogenicity, mature DCs might not always induce immunity. Hence, other factors such as exposure to certain differentiation signals or cues from the local environment might condition DCs beyond their expression of co-stimulatory molecules. Therefore, tolerance is not only a consequence of T cells receiving insufficient signals 2 and 3, but additional tolerance inducing factors might be in play.

GENERATION OF TOLEROGENIC DCs

T_{reg} CELLS, THROUGH VARIOUS MECHANISMS, PROMOTE THE GENERATION OF TOLEROGENIC DCs

While several environmental factors and cytokines can promote the tolerogenic phenotype of DCs, there is accumulating evidence that T_{reg} cells can also induce a tolerogenic phenotype in DCs by modulating their maturation and function. Several studies indicate that T_{reg} cells can suppress the capacity of DCs to activate T_{eff} cells by down-regulating CD80/CD86 expression on bone marrow-derived or splenic DCs *in vitro* (Cederbom et al., 2000). Depletion of T_{reg} cells from asthma susceptible mice resulted in DCs with higher expression levels of MHC II, CD80, CD86 and displayed a increased T cell stimulatory activity (Mahnke et al., 2007). Although the mechanism by which T_{reg} cells achieve this is unknown, this might be mediated through cell surface molecules or cytokines such as IL-10, CTLA-4, and TGF- β (Figure 1).

Role of T_{reg} cell-derived IL-10 in induction of tolerogenic DCs

The production and activity of the immunomodulatory cytokine IL-10 is often associated with tolerogenic responses. IL-10 inhibits multiple aspects of DC function including MHCII and CD80/CD86 co-stimulatory molecules expression and a release of pro-inflammatory cytokines such as IL-1 β , IL-6, TNF- α , and IL-12 (Mahnke et al., 2002, 2007; Alexander, 2005). Interestingly, IL-10 mediated effects could be only observed when immature DCs were exposed to IL-10 (Read et al., 2000), as mature DCs were insensitive to IL-10 stimulation and displayed stable, mature phenotype (Read et al., 2000). In addition, DCs cultured in presence of T_{reg} cells has been shown to secrete of IL-10, TGF- β , and IL-27 cytokines (Awasthi et al., 2007). IL-27 cytokine plays an important role in suppressing production of Th17 polarizing cytokines, such as IL-1 β , IL-6, IL-23 derived from DCs and act on naïve T cells to induce Tr1 differentiation (Pot et al., 2009). IL-10 treated DCs have also been shown to promote tumor growth, and prevent transplant rejection and ameliorate MS (Mahnke et al., 2007).

CTLA-4 is a potent inducer of tolerogenic DCs

CTLA-4 plays an important role in T_{reg} cell-mediated suppression (Salomon et al., 2000). T_{reg} cells constitutively express high levels of CTLA-4 and conditional deficiency of CTLA-4 in T_{reg} cells results in lymphoproliferation and variety of autoimmune disease, an outcome analogous to global CTLA-4 deficient mice (Wing et al., 2008). Monoclonal antibody blockade of CTLA-4 exacerbates T1D in non-obese diabetic (NOD) mice and induce IBD (Read et al., 2000; Wing and Sakaguchi, 2010). CTLA-4 mediates suppression through down regulation of CD80 and CD86 expression on DCs. T_{reg} cells from CTLA-4 deficient mice or

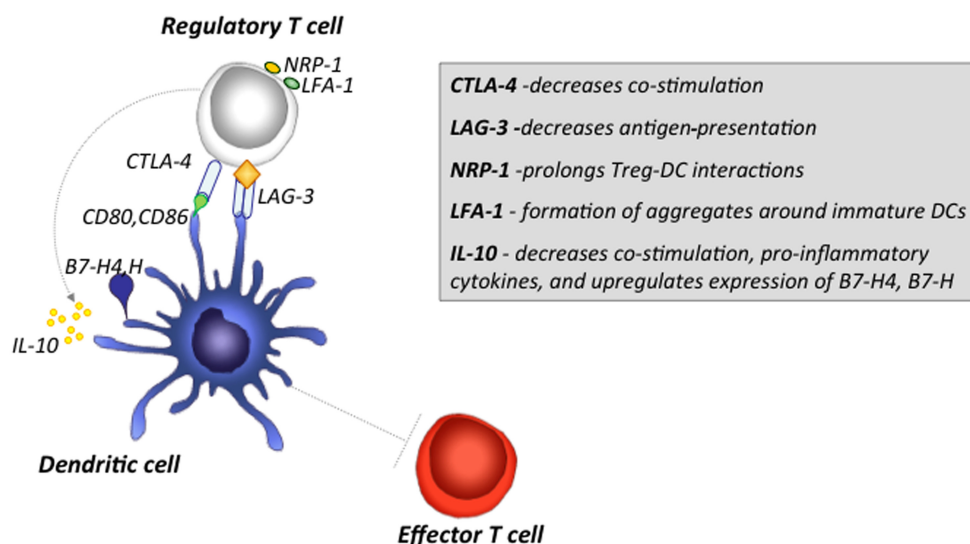


FIGURE 1 | Major mechanisms by which T_{reg} cells induce tolerogenic DCs and inhibit T_{eff} cell activation. T_{reg} cells can inhibit the function of DCs through various mechanisms. CTLA-4 expression on T_{reg} cells down-regulates up-regulation of CD80 and CD86 co-stimulatory molecules on DCs. LAG3 binding to MHCII-expressing immature DCs results in inhibitory signals that suppress DCs maturation and co-stimulatory capacity. Nrp-1 expression on T_{reg} cells promotes sustained interactions between

T_{reg} cells and DCs, and limits the access of T_{eff} cells to DCs. Expression of LFA-1 on T_{reg} cells, promotes aggregate formation around DCs, and inhibit the up-regulation of CD80 and CD86 on DCs. IL-10 inhibits up-regulation of MHCII and B7 co-stimulatory molecule expression, suppresses release of pro-inflammatory cytokines such as IL-1 β , IL-6, TNF- α , and IL-12, and up-regulates of B7-H4 and B7-H inhibitory molecules.

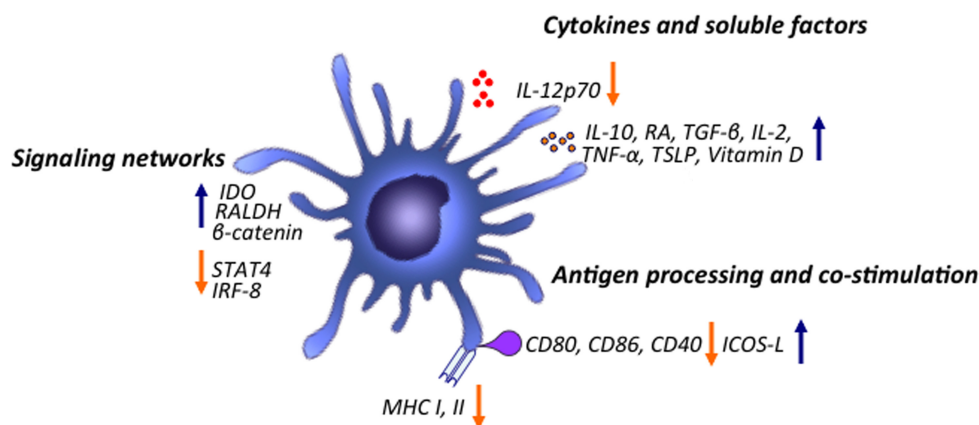


FIGURE 2 | Major mechanisms of immune modulation by tolerogenic DCs. DCs modulate the immune system by promoting T_{reg} cell generation through various mechanisms. Upon stimulation, DCs are able to increase production of various cytokines and inflammatory mediators such as IL-10, RA, vitamin D, TGF- β , IL-2, IL-10, TSLP, TNF- α and down-regulate expression of inflammatory cytokines such as IL-12p70. DCs, by down-regulating co-stimulation and antigen presentation, can favor induction of T_{reg} cells. Various signaling pathways program DCs to induce tolerogenic responses.

Activation of β -catenin signaling pathway promotes induction of anti-inflammatory factors such as vitamin A and is important for promoting T_{reg} cell induction and limiting inflammatory responses. TSLPR signaling inhibits activation of STAT4 and IRF-8, factors critical for production of the Th1 polarizing factor IL-12. Triggering of DCs through TLR2-6 leads to Erk activation which mediates induction of RALDH and conversion of retinal to RA. TLR9 ligation can drive induction of IDO in DCs limiting IL-6 production and differentiation of Th17 cells while inducing differentiation of T_{reg} cells.

administration of CTLA-4 blocking antibodies results in reduced down-modulation of B7 molecules and increased T cells proliferation (Oderup et al., 2006). IDO, a potent regulatory molecule that is known to induce the production of pro-apoptotic metabolites from the catabolism of tryptophan, results in the suppression of T_{eff} cells through a mechanism dependent on interaction between CTLA-4 and CD80/CD86 (Fallarino et al., 2003; Baban et al.,

2009). Baban et al. (2009) recently demonstrated that IDO is a critical molecular switch that stimulates potent T_{reg} cells suppression while simultaneously block IL-6 driven reprogramming of T_{reg} cells into Th17 cells and production pro-inflammatory cytokines such as IL-17, IFN- γ , TNF- α , and IL-2. Interestingly, it has also been demonstrated that T_{reg} cells can modulate DCs to express IDO and more specifically CTLA-4 immunoglobulin

fusion protein was found to induce IDO expression through ligation to CD80 and CD86 molecules in mouse and human DCs (Fallarino et al., 2003; Mellor and Munn, 2004; Mellor et al., 2004). In addition, IL-10 produced by T_{reg} cells is critical for sustaining IDO expression in DCs (Manicassamy and Pulendran, 2011). Thus, IDO is one of the molecular switches controlling the balance between T_{reg} and T_{eff} cell responses.

B7-H4 and B7-H: B7 family members that negatively regulate T cell immunity

A number of other mechanisms have been proposed by which T_{reg} cells can either abrogate the antigen presenting capacity of DCs or promote the secretion immunomodulatory cytokines. For example, T_{reg} cells are able to induce the expression of B7-H and B7-H4, a ligands responsible for negative regulation of cell-mediated immunity in peripheral tissues (Mahnke et al., 2007). Recently, T_{reg} cells have also been shown to trigger high levels of IL-10 production by APCs and in turn stimulate B7-H4 expression in an autocrine fashion and render these APCs immunosuppressive (Kryczek et al., 2006).

Lymphocyte activation gene 3 (LAG3)

Lymphocyte activation gene 3 is cell surface molecule expressed on T_{reg} cells that can modulate DC phenotype and function (Huang et al., 2004; Workman and Vignali, 2005; Liang et al., 2008). LAG3 is a CD4 homolog that binds MHC II molecules with very high affinity, has a negative regulatory intrinsic function and is required for maximal T_{reg} cells suppression (Huang et al., 2004; Workman and Vignali, 2005). Binding of LAG3 to MHC II molecules on immature DCs initiates a receptor tyrosine-based activation motif (ITAM) mediated inhibitor signaling pathway that suppress DC maturation (Liang et al., 2008). These findings provide a novel tolerogenic pathway that may endow T_{reg} cells to enhance tolerance by inhibiting DCs functions.

T_{reg}-DCs cognate interactions mechanism

It has been shown that T_{reg} cells are more motile than naïve T cells *in vitro*, in turn out-competing the latter for physical access to DCs (Tran et al., 2009). This T_{reg}-DC cell aggregation process is antigen-dependent, and the selective advantage of T_{reg} cells over T_{eff} cells for physical interactions with DCs can be attributed, at least in part, to the expression of LFA-1 (Lymphocyte function-associated antigen 1) by T_{reg} cells, as deficiency or blockade of LFA-1 abolishes this process. By forming aggregates, T_{reg} cells inhibit the up-regulation of CD80, CD86 on immature DCs and also down-regulate the expression of CD80 and CD86 by mature DCs without affecting the expression of CD40 or MHC II molecules (Onishi et al., 2008). This modification of CD80 and CD86 is CTLA-4 dependent as T_{reg} cells from CTLA-4 deficient mice fail to modify expression of CD80 and CD86 (Onishi et al., 2008). Therefore at least *in vitro*, T_{reg} cell contact dependent suppression can be dissected by two consequent steps; initial LFA-1 dependent formation of aggregates around immature DCs and subsequent LFA-1 and CTLA-4 dependent active down-regulation of CD80 and CD86 expression (Onishi et al., 2008). Overall, this results in down-regulation of T_{eff} cell activation and induction of immune suppression and tolerance. The significance of DCs and T_{reg} cells interaction has been also demonstrated in studies

using NOD mice. The Bluestone group, by means of intravital microscopy, demonstrated that direct interactions between T_{reg} cells and DCs *in vivo* resulted in inhibition of T_{eff} cells activation (Tadokoro et al., 2006; Tang et al., 2006) suggesting a potential feedback loop between T_{reg} cells and DCs. In this model, T_{reg} cells up-regulate LFA-1 expression upon activation by immature DCs, adhere to DCs, and suppress the expression of CD86 and CD80, in turn inhibiting the activation and expansion of T_{eff} cells (Sakaguchi et al., 2009). While Foxp3⁺ T_{reg} must be activated by antigen in order to exert their suppressive functions *in vitro* and *in vivo* (Takahashi et al., 1998), activated Foxp3⁺ T_{reg} cells can mediate *in vitro* bystander suppression of T_{eff} cells with different antigen specificities. However, it still remains to be determined whether such mechanism can also apply to other cell types like DCs, and to what extent such bystander suppression occurs *in vivo*.

Recently it has been shown that neuropilin (Nrp-1) also plays a critical role in mediating T_{reg}-DC interactions (Sarris et al., 2008). Nrp-1 is a ligand binding receptor for a class of semaphorins, and is preferentially expressed by T_{reg} cells. Nrp-1 expression can be induced by ectopic expression of Foxp3 in Foxp3⁻ T cells, and antibody blockade of Nrp-1 reduces the frequency of T_{reg}-DCs interactions. Furthermore, retroviral introduction of Nrp-1 endows T helper cells with ability to establish a long interactions with DCs (Sarris et al., 2008). Interestingly, it has been demonstrated that Nrp-1 can confer a high adhesive property of T_{reg} cells in their interaction with DCs under steady conditions; however, this potential by T_{reg} cells is lost under inflammatory conditions (Sarris et al., 2008).

The results described above demonstrate the capacity of Foxp3⁺ T_{reg} cells, through the expression of LAG3 and Nrp-1 molecules, to influence immature but not mature DCs. Mature DCs, in contrast to immature DCs, produce pro-inflammatory cytokines such as IL-6, TNF- α , and IL-1 β which can down-regulate Foxp3 expression and abolish Foxp3⁺ T_{reg} cell-mediated suppression (Rutella et al., 2006). Although IL-6 production induced by LPS stimulation only slightly reduced Foxp3⁺ T_{reg} cells-mediated suppression, DC-derived IL-6 may render responder T cells resistant to suppression (Pasare and Medzhitov, 2003). Moreover, IL-6 might also facilitate the reprogramming of Foxp3⁺ T_{reg} cells into Th17 lineage (Bettelli et al., 2007).

In other instances, it has been demonstrated that T_{reg} cells, through the expression of LFA-1 and CTLA-4, can suppress TNF and LPS matured DCs by selectively down-regulating CD80, CD86, PDL1, and PDL2, but not MHCII and CD40, expression on the DC surface. Overall, these findings demonstrate that T_{reg} cells may employ specific mechanism(s) to suppress DCs functions in different microenvironments of antigen priming.

THE GUT ENVIRONMENT PROMOTES DEVELOPMENT OF TOLEROGENTIC DCs

The identification of tolerogenic DCs subsets in microenvironments such as gut, skin, and lungs suggest that local signals induce tolerogenic DCs *in situ*. Given the enormous amount of microbial stimuli in the gut, intestinal DCs represent a key regulatory mechanism to prevent excessive inflammation. Specifically, CD11c⁺ DCs expressing CD103 have been identified in the gut-associated lymphoid tissue (GALT) and mesenteric lymph node with the

specialized function of inducing Foxp3⁺ T_{reg} cells and maintaining gut immune homeostasis (Coombes et al., 2007; Sun et al., 2007). This CD11c⁺ DC subset expresses high levels of CD103, MHC II, CD80, and CD86. Interestingly, absence of CD103 on DCs led to the abrogation of T_{reg} cell activity indicating a crucial role for CD103 in maintaining the balance between T_{reg} and T_{eff} cells (Annacker et al., 2005). While mesenteric lymph node derived CD103⁺ DCs are prone to imprint expression of CCR9 on T cells, an important homing receptor enabling homing to the gut, CD103⁻ DCs promote the differentiation of CD4⁺ T cells producing IFN- γ (Jaensson et al., 2008). Collectively, CD103⁺ and CD103⁻ DCs represent functional subsets and CD103 is critical to regulate T_{eff} and T_{reg} cells in the gut (Annacker et al., 2005).

APOPTOTIC DCs FAVOR FORMATION OF TOLEROGENIC DCs

Recent studies show that immature DCs can uptake apoptotic and necrotic DCs without being recognized as an inflammatory event (Kushwah et al., 2010). This uptake results in conversion of mature DCs into tolerogenic DCs that remain resistant to LPS induced maturation and induces the production of TGF- β via the mTOR signaling pathway (Kushwah et al., 2010). TGF- β producing DCs subsequently interact with naïve T cells and drive the differentiation of iT_{reg} cells (Kushwah et al., 2010; Kushwah and Hu, 2011). Moreover, recent studies showed that α -CD3 mAb treatment transiently depletes large numbers of T cells and induce long term immune tolerance (Perruche et al., 2008; Esplugues et al., 2011). The mechanism underlying this regulatory outcome is due to increased production of TGF- β by immature DCs after engulfment of apoptotic T cells (Perruche et al., 2008). A recent study demonstrated that α -CD3 mAb treatment resulted in elimination of the inflammatory Th17 cells from intestinal lumen or resulted in acquisition Th17 cells producing a IL-10 (Esplugues et al., 2011). However, it is not known which specific subset of gut-residing DCs is responsible for production of TGF- β and induction of IL-10 producing Th17 cells with regulatory capacity.

IMMUNE MODULATION BY TOLEROGENIC DCs

DC EMPLOYS VARIOUS MECHANISMS TO DRIVE THE DIFFERENTIATION OF T_{reg} CELLS

B7 co-stimulatory molecules promote differentiation of T_{reg} cells

In the steady state, DCs are largely immature and present antigens to T cells in tolerogenic manner. Such DCs are characterized by low expression of CD80, CD86, and CD40 (Steinman and Nussenzweig, 2002; Steinman et al., 2003; Hubert et al., 2007). Moreover, targeting of antigens to immature DCs via the regulatory receptor DEC205 results in tolerance through deletion of antigen-specific T cells and preferential induction of T_{reg} cells (Hawiger et al., 2001). Interestingly, antibodies bound to DEC205 are efficiently internalized and delivered to antigen processing compartments, however internalization of the antigen by DEC205 does not induce the maturation of the DCs (Mahnke et al., 2000). Immature DCs had been shown to induce T_{reg} cells *in vitro* and anti-DEC205 targeting of antigens to immature DCs led to anergic T cells *in vivo* (Mahnke et al., 2003). These data show that that the DEC205 receptor is critical for DCs in the steady state

to promote tolerance (Mahnke et al., 2003). Moreover, treatment with respective anti-DEC antigen conjugates results in significant improvement of autoimmune disorders such as T1D and MS (Hawiger et al., 2004; Bruder et al., 2005). DCs can also favor tolerance by utilizing ICOS, another CD28 family member, to specifically drive Tr1 cell differentiation (Akbari et al., 2002; Ito et al., 2007).

Despite the established role of immature DCs as inducers of T_{reg} cells, recent studies have shown that mature DCs, expressing high levels of CD86, also have the potential to preferentially expand T_{reg} cells *in vitro*, and prevent T1D development (Yamazaki et al., 2003; Tarbell et al., 2004; Sgouroudis et al., 2011). Interestingly, DCs isolated from Peyer's patches, eye, or lungs display a mature phenotype, secrete IL-10, but not IL-12 and drive the development of Tr1 cells (Akbari et al., 2001). Thus, signals derived from the local tissue environment might play a role in conditioning tolerogenic DCs and drive the differentiation of T_{reg} cells. All together, these findings suggest that the previously established paradigm whereby immature DCs leads to T_{reg} cell differentiation and mature DCs drive T_{eff} cell responses might be revisited (Figure 2).

DC-DERIVED CYTOKINES THAT DRIVES DIFFERENTIATION OF T_{reg} CELLS

IL-10

Dendritic cells can secrete high amounts of IL-10 upon stimulation and drive differentiation of naïve T cells into IL-10 secreting Tr1 cells (Kushwah and Hu, 2011). DCs of IL-10 transgenic mice display a particularly immature phenotype and Tr1 cells are significantly enriched in spleens of these mice (Wakkach et al., 2003). Langerhans cells in the skin also produce IL-10 and drive differentiation of Tr1 cells (Igyarto et al., 2009).

TNF- α

TNF- α can also induce tolerogenic state in DCs (Menges et al., 2002; Mahnke et al., 2007). Despite the fact that TNF- α treated DCs has a mature phenotype, they fail to secrete inflammatory cytokines such as IL-1 β , IL-6, TNF- α , and IL-12 (Menges et al., 2002). Moreover, these tolerogenic DCs were able to reverse development of EAE where their suppressive effects were mediated by the induction of IL-10 producing T_{reg} cells (Mahnke et al., 2007). However, there is also a controversial data demonstrating that TNF- α blockade prevented DC maturation and T_{reg} cells were induced in the absence of TNF- α (Mahnke et al., 2007).

TGF- β

TGF- β -producing DCs might preferentially drive differentiation of T_{reg} cells rather than T_{eff} cells. Tumor-bearing mice contains a CD11c⁺ DC subset characterized by low expression of CD80 and CD86 co-stimulatory molecules and are endowed with the capacity to secrete TGF- β which promotes T_{reg} cells differentiation and proliferation *in vivo* (Ghiringhelli et al., 2005).

IL-2

We and others have shown that a local deficiency in islets of IL-2, a critical cytokine for the homeostasis/fitness of T_{reg} cells *in vivo*, compromises T_{reg} cell function in islets, a defect readily

corrected by low dose IL-2 therapy in NOD mice. NOD mice introgressed with a protective *IL2* allelic variant from T1D-resistant C57BL6 mice (NOD.Idd3^{BL6}) are resistant to autoimmune diabetes in contrast to wild-type NOD mice which are susceptible to the disease. Moreover, T1D-protective *IL2* allelic variants in NOD mice impinge T1D development by bolstering IL-2 production by CD4⁺ T_{eff} cells in turn, driving the expansion and homeostasis of CD4⁺Foxp3⁺ T_{reg} cells in islets. Recently, we showed that CD11c⁺ DCs in NOD.Idd3^{BL6} have an increased maturation status relative to wild-type NOD CD11c⁺ DCs. We also showed that NOD.Idd3^{BL6} DCs are more potent activators of T_{reg} cells functions *in vitro* and *in vivo*, and this increased capacity of congenic DCs to prime T_{reg} cells is attributed to their ability to produce IL-2. Consistently, IL-2 blockade *in vitro* completely abolished the proliferative advantage conferred by Idd3^{BL6} DCs on Foxp3⁺ T_{reg} cells (Sgouroudis et al., 2011). Interestingly, CD11c⁺ MHCII⁺ DCs isolated from pancreatic LN of NOD.Idd3^{BL6} mice promoted Foxp3⁺ T_{reg} cells expansion more efficiently than WT DCs isolated from similar sites, suggesting that Idd3^{BL6} DCs display tolerogenic phenotype specifically in the pancreatic sites to enhance Foxp3⁺ T_{reg} cells functions (Sgouroudis et al., 2011). Thus, T1D-protective *IL2* allelic variants impinge the development of β -islet autoimmunity by bolstering IL-2 mRNA expression and protein secretion by CD4⁺ T_{eff} cells and DC and in turn, driving the functional homeostasis of CD4⁺Foxp3⁺ T_{reg} cells in the target organ (Sgouroudis et al., 2011).

It has been previously shown that CD40/CD40L interaction regulates T_{reg} cells homeostasis (Kumanogoh et al., 2001; Guiducci et al., 2005). Mature DCs lacking CD40L are impaired in sustaining T_{reg} cells proliferation and survival. The underlying mechanism is mediated by fact that CD40L deficient DCs are not able to produce IL-2, to as similar extent as wild-type DCs. Administration of rhIL-2 *in vivo* restored T_{reg} cells numbers in thymic and peripheral compartments of CD40L deficient mice, by increasing survival and homeostatic proliferation of T_{reg} cells (Kumanogoh et al., 2001; Guiducci et al., 2005). Therefore, CD40 triggering by T_{reg} cells contributes to induce IL-2 through CD40L on DCs needed for their maintenance. Therefore, several mechanisms have been established by DCs in order to maintain a peripheral pool of T_{reg} cells through IL-2 production (Kumanogoh et al., 2001; Guiducci et al., 2005).

IDO EXPRESSION BY DCs DRIVES T_{reg} CELLS DIFFERENTIATION

Dendritic cells populations expressing IDO play a critical role in immune tolerance by promoting iT_{reg} cells differentiation (Mellor and Munn, 2004; Matteoli et al., 2010). IDO expression by DCs appears to be dependent on Aryl hydrocarbon receptor (AHR) as DCs lacking AHR fail to up-regulate IDO and prime T cells responses rather than tolerance induction (Nguyen et al., 2010). Selective inhibition or genetic deletion of IDO affects the development of antigen-specific T_{reg} cells while promotes Th1 and Th17 development and worsens T cells mediated and dextrin sulfate sodium (DSS) colitis in mice (Matteoli et al., 2010). It has been shown that TLR9 ligation can drive induction of IDO in pDCs that subsequently suppress IL-6 production and differentiation of Th17 cells while induce differentiation of T_{reg} cells (Baban et al., 2009).

SITE SPECIFIC RESIDING DCs SUPPORTS T_{reg} CELLS DIFFERENTIATION Thymic residential DCs play a role in induction of Foxp3⁺ T_{reg} cells

It has been shown that DCs and medullary thymic epithelial cells (mTECs) contribute to the selection of Foxp3⁺ T_{reg} cells in the thymus. Epithelial cells in Hassall's corpuscles in the thymus produce thymic stromal lymphopoietin (TSLP) which subsequently acts on thymic DCs by binding to TSLP receptor (TSLPR) and IL-7R α complex and drives induction of CD80 and CD86 (Ziegler and Liu, 2006). These DCs subsequently drive differentiation of CD4⁺CD8⁺CD25⁻ thymocytes into Foxp3⁺T_{reg} cells, which highly depend from IL-2 and CD28 signaling (Ziegler and Liu, 2006). These data demonstrate that TSLP activated myeloid DCs are critical for selection of self-reactive thymocytes to develop into Foxp3⁺ T_{reg} cells. In addition to the myeloid DCs, human thymic pDCs can also induce differentiation of T_{reg} cells in a CD40L-dependant fashion (Hanabuchi et al., 2010; Martin-Gayo et al., 2010). Thymic pDCs express TSLPR along with IL-7R α and are responsive to TSLP derived from thymic epithelial cells (Hanabuchi et al., 2010; Martin-Gayo et al., 2010). More evidence for the contribution of thymic DCs in T_{reg} cell induction comes from studies done in mouse models, where targeting of neo-self antigens to thymic DCs instructed antigen-specific T cells to develop into Foxp3⁺ T_{reg} cells (Proietto et al., 2008). Three thymic DCs subtypes, namely conventional DCs, thymus resident DCs, and pDCs were shown to be able of instructing Foxp3⁺ T_{reg} cells differentiation *in vitro*, supporting the model where antigen recognition on thymic DCs can directly elicit Foxp3⁺ T_{reg} cells differentiation (Wu and Shortman, 2005).

Gut-residing DCs maintain T_{reg} cell stability via production of retinoic acid and TGF- β

Dendritic cells from the lamina propria of the small intestine preferentially promote T_{reg} cell induction relative to the DCs of lymphoid organs. This increased induction of T_{reg} cells has been shown to be dependent on TGF- β and retinoic acid (RA), a vitamin A metabolite highly expressed in small intestine (Coombes et al., 2007; Sun et al., 2007; Belkaid and Oldenhove, 2008). DCs are the major subset of immune cells able of metabolizing vitamin A to RA. RA synthesis is a highly regulated process that involves several key enzymes: vitamin A is oxidized to retinaldehyde by alcohol dehydrogenase and then to RA by retinal dehydrogenase (RALDH). Manicassamy et al. (2009) and Manicassamy and Pulendran (2011) demonstrated that TLR2 and TLR6 signaling by zymosine promote induction of RALDH1 and RALDH2 in DCs via ERK dependent mechanism. This result in conversion of retinal to RA which then exerts an autocrine effect on DCs via RA receptor to induce SOCS3, this then suppress activation of p38MAPK and pro-inflammatory cytokines. Accumulating evidence shows that RA directly influences the development and function of various immune cells. For example, RA promotes the differentiation of T_{reg} cells and suppress differentiation of Th1 and Th17 cells, and prevents IBD and EAE in mice (Kushwah and Hu, 2011). CD103⁺ DCs in the intestine lamina propria and mesenteric lymph nodes express high levels of RALDH and have ability to induce Foxp3⁺ T_{reg} cells. It is well documented IL-6 is an essential cytokine driving Th17 cell differentiation, while down-regulate

Foxp3 expression in T_{reg} cells. Interestingly, Zhou et al. (2010) showed that RA maintains the stability of Foxp3⁺ T_{reg} cells and sustains their suppressive potential in the presence of IL-6, through down-regulation of IL-6 receptor expression and signaling, therefore preventing T_{reg} cells conversion into Th17 cells (Coombes and Powrie, 2008; Zhou et al., 2010). CD103⁺ DCs from the lamina propria of the small intestine and mesenteric lymph node have been shown to be significantly better than splenic DCs at mediating the conversion of naïve T cells into Foxp3⁺ T cells in the presence of exogenous TGF- β (Coombes et al., 2007). Moreover, expression of the $\alpha_v\beta_8$ integrin by DCs is important for TGF- β activation, accumulation of T_{reg} cells in the small intestine and prevention of colitis development (Coombes and Powrie, 2008). Overall, gut-derived signals may condition DCs to produce TGF- β for T_{reg} cell generation.

β Catenin: a novel regulator of intestinal homeostasis

Wnt- β -catenin signaling in intestinal DCs regulates the balance between inflammatory and regulatory responses. Recently, Manicassamy et al. (2009) performed a gene expression profile of lamina propria DCs and demonstrated that several Wnt family genes and β -catenin were constitutively expressed by intestinal DCs, but not by splenic DCs (Manicassamy et al., 2009). Furthermore, they showed that β -catenin signaling promotes the induction of T_{reg} cells while suppress Th1 and Th17 cells in the gut, indicating that β -catenin expression by intestinal DCs is important for maintaining the balance between T_{reg} and T_{eff} cells in the gut (Manicassamy et al., 2010). In addition, intestinal DCs lacking β -catenin expression produced lower levels of T_{reg} cell-promoting stimuli including RA metabolizing enzymes, IL-10, and TGF- β , but higher levels of Th17-promoting cytokines IL-23 and IL-6 (Manicassamy et al., 2010). Overall, β -catenin signaling is needed to maintain intestinal homeostasis through the induction of T_{reg} cells and the suppression of pro-inflammatory factors.

The gut microenvironment influences intestinal DC function

Many of the unique properties of intestinal DCs appear to be a result of environmental conditioning. Activation of NF- κ B expression in intestinal epithelial cells, perhaps as a result of microflora signaling through PRRs, enhances TSLP production (Coombes and Powrie, 2008). Recently, important role for TSLP in dictating the quality of immune response has been suggested. TSLP and other epithelial cells factors limits the activation of STAT4 and IRF-8, essential factors for the production of Th1 polarizing cytokine IL-12-23p40 (Arima et al., 2010). In addition, TSLP signaling also induce the activation of STAT6, which programs DCs to secrete chemokines necessary for the recruitment of Th2 cells, and increase IL-10 and TGF- β production (Coombes and Powrie, 2008; Arima et al., 2010). TSLP has also been shown to confer human thymic DCs with ability to induce the differentiation of CD4⁺CD25⁻ thymocytes into Foxp3⁺CD4⁺CD25⁺T_{reg} cells (Watanabe et al., 2005). Thus, the increased ability of gut-residing CD103⁺ DCs to induce Foxp3 may be due to TSLP.

Overall, these findings illustrate that the intestinal immune system (i.e., GALT) is a preferential site for the induction of T_{reg} cells, and provides a mechanism by which the thymus-derived T_{reg} cell pool could be complemented by *de novo* generated T_{reg}

cells for the efficient control of inflammatory responses toward specific commensal bacteria and food antigens (Coombes and Powrie, 2008).

The skin and lung are naturally tolerogenic environments

Like the intestine, the skin and lungs are also constantly exposed to various microbes and environmental antigens such as allergens. The interaction between vitamin D and RANK–RANKL signaling pathway in the skin plays a role in DC-mediated iT_{reg} cells induction (Loser et al., 2006; Kushwah and Hu, 2011). This process occurs *via* the activated metabolite of vitamin D, VD3, which exerts its actions through its nuclear receptor, VDR. VDR is expressed on DCs and vitamin D treatment inhibits DCs maturation and their ability to prime alloreactive T cells responses (Loser et al., 2006). Similarly to the intestine, lung CD103⁺ DCs promote the induction of T_{reg} cells, while the CD103⁻ DCs represent the main producers of pro-inflammatory cytokines in response to airborne allergens and TLR ligands. In contrast to lung and mucosal sites, CD103⁻CD11b⁺ migratory dermal DCs, compared to CD103⁺CD11b⁺ DCs, are much more potent in promoting T_{reg} cells induction (Guilliams et al., 2010; Pulendran et al., 2010).

THERAPEUTIC APPLICATIONS OF TOLEROGENTIC DCs

The possibility to generate or expand tolerogenic DCs *in vitro* provides significant opportunities for therapeutic interventions. DCs are generated *in vitro* from bone marrow precursors in rodents or blood monocytes in humans and can be rendered tolerogenic by modulating their culture conditions through exposure to cytokines, growth factors or pharmacologic mediators, or genetic engineering (Morelli and Thomson, 2007). *In vitro* generation of tolerogenic DC can be achieved through exposure to various anti-inflammatory agents such as vitamin D, as well as clinically approved suppressive drugs such as corticosteroids, cyclosporin, and rapamycin (Morelli and Thomson, 2007). Rapamycin acts as an inhibitor of the Akt–mTOR pathway, increases the number of Foxp3⁺ T_{reg} cells, and promotes their resistance to apoptosis (Strauss et al., 2007; Basu et al., 2008). Moreover, rapamycin-conditioned myeloid DCs fail to produce IL-12p70 and TNF- α and are resistant to maturation induced by TLR ligands or by CD40 signaling (Turnquist et al., 2007). Advances in gene transfer technology offers the possibility to generate tolerogenic DCs by genetically inducing the expression of immunosuppressive molecules like IL-10, TGF- β , or CTLA-4, or blocking the expression of co-stimulatory molecules (Morelli and Thomson, 2007). Conversely, it is possible to generate tolerogenic DCs *in vitro* that drive T_{reg} cells differentiation in a tissue specific manner in order to inhibit inflammation in a particular site (Rescigno, 2010). For example, intestinal epithelial cells release factors that drive the development of mucosal like tolerogenic DCs (Iliev et al., 2007). Incubation of DCs with intestinal, but not mammary or epithelial cell-derived supernatants induces the expression of CD103 on DCs while inhibiting the secretion of inflammatory cytokines. These *in vitro* generated CD103⁺ DCs can drive the induction of T_{reg} cells and expression of the gut-associated homing receptor $\alpha_4\beta_7$ (Iliev et al., 2009). Moreover, only intestinal epithelial cells conditioned DCs are able to protect against colitis development (Iliev et al., 2009). Therefore, DCs conditioned at local environment are

important for generation of T_{reg} cells that are able to suppress and home to specific sites.

CONCLUSION

Our understanding of the functional and phenotypic plasticity of DCs, as well the capacity to modulate DCs development and maturation *in vitro* and *in vivo* gives opportunity to use these cells for therapeutic purpose in autoimmunity and cancer. Tolerogenic DCs has a dual role, for example in cancer, a profound defect in DCs function are associated with accumulation of immature DCs in tumors where DCs are unable to initiate anti-tumor immune responses while contributes to the recruitment, expansion and function of T_{reg} cells. While this have negative outcome in cancer settings, similar scenario would be helpful in the case of autoimmunity. Several studies in mice suggest that DCs might be used in the treatment of autoimmunity through their ability to induce T_{reg} cells. For example, repetitive injections of semi-mature DCs results in protection from EAE and

thyroiditis (Ueno et al., 2007). In the NOD mice, DC-mediated *in vitro* generation of T_{reg} cells can inhibit spontaneous T1D development in these mice (Ueno et al., 2007). In recent years, several mechanisms have been identified to explain how tolerogenic DCs mediate their function. For example, IDO expression by intestinal DCs is important for regulating intestinal immune homeostasis by keeping the balance between T_{reg} cells and Th17, Th1 cells. Deregulations of IDO activity results in increased intestinal inflammation, suggesting that IDO could be regarded as a new target for IBD. Therefore, pharmacological targeting of IDO during the chronic inflammation like IBD would be beneficial in dampening the inflammatory process and tissue damage in the gut (Matteoli et al., 2010). Moreover, as IL-6 is often present in inflammatory infiltrates in autoimmune settings, recent findings about ability of RA to stabilize T_{reg} cells in the presence of IL-6 offers the possibility that RA treated T_{reg} cells can be used in many autoimmune settings where achieve such a balance is essential.

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